Comparison of staphylococcal specific and non-specific adhesion to adsorbed fibronectin

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Statement of Purpose: Nosocomial infections are the fifth leading cause of death in the U.S.[1] The overwhelming majority of these infections are caused by staphylococci, particularly *Staphylococcus epidermidis (SE)* and *Staphylococcus aureus (SA),* opportunistic pathogens that adhere to the surfaces of implanted medical devices in the blood stream. *SA* binds to surfaces through specific adhesion mechanisms to blood proteins such as fibronectin (FN) while *SE* is thought to bind by both specific adhesion to blood proteins and via non-specific adhesion to plastics. Upon adhering, *Staphylococci* form biofilms, 3-D structures that obviate the efficacy of both antibiotics and the normal immune system. Once a biofilm has formed, the implant must be removed to prevent further infection and death. Preventing adhesion of *Staphylococci* to medical devices is a crucial first step in averting biofilm infections. However, negating this adhesion requires an understanding of the bonds between the cell receptor and surface ligands involved in adhesion. While *SA* specific adhesion has been well described previously, the mechanism of *SE* adhesion to blood proteins such as FN remains unclear.

Methods: *Staphylococcus aureus 12600* and *Staphylococcus epidermidis 1457* were grown in Tryptic Soy Broth (BD) overnight and inoculated into new media for 4 hours until late exponential growth. Cells were rinsed twice in phosphate buffered saline (PBS) and resuspended at 10^9 cells/ml for adhesion experiments. Varying concentrations of human serum fibronectin (Sigma) or Bovine Serum Albumin were adsorbed to tissue culture polystyrene (TCPS) surfaces, 96 well plates (Corning) for static experiments or 35mm dishes (Corning) for flow adhesion studies, overnight at 4°C and rinsed twice. To examine the specificity of *SE* binding to FN binding, staphylococcal cells were incubated on FN or BSA coated surfaces that had either been coated with a 1% BSA solution to block available TCPS substrata or remained unblocked. Cells were stained with Syto9 nucleic acid stain (Invitrogen) and 100µl of cell solution was added to each well and incubated for 2 hours at 37°C. Wells were rinsed twice and the number of adherent cells was determined on a fluorescent plate reader using a standard curve of a known number of cells to calibrate fluorescence. To examine the strength of binding to the protein coated surfaces, dynamic adhesion and detachment experiments were performed. Cell suspensions were delivered over protein-coated TCPS substrates at a low shear rate ($10s^{-1}$), allowed to settle for 15 minutes and were subjected to step-wise increments of increasing shear (from $10s^{-1}$ to $1000s^{-1}$) to remove weakly bound cells. All experiments were performed in triplicate. Statistical analysis was performed using Student's t-test.

Results: In blocked static adhesion experiments, *SE* specific adhesion to FN was not significantly increased over BSA adhesion. However, in unblocked adhesion studies, a dose dependent decrease in adhesion occurred with increasing concentrations of either FN or BSA (Figure 1). Due to the specific nature of *SA* adhesion to FN, this decrease in FN binding did not occur in *SA* adhesion experiments (Figure 2). *SE* adhesion to both FN and BSA was similar to the low level non-specific adhesion of *SA* to BSA, decreasing with increasing protein concentration on the surface.

This weak *SE*:FN binding was confirmed in dynamic adhesion and detachment experiments. *SE* bound to either FN or BSA, detached much more readily from surfaces than *SE* bound to the substratum alone.

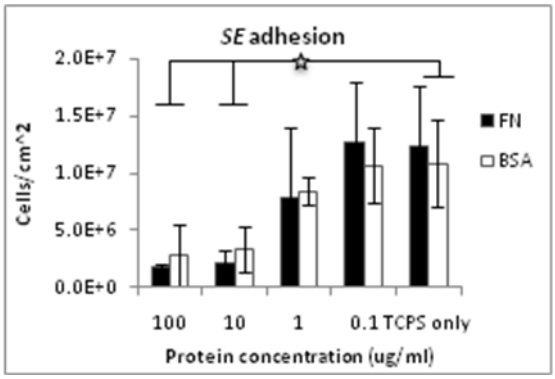

Figure 1. *SE* binding to FN and BSA via non-specific adhesion. * indicates significant ($p<0.05$) difference between FN or BSA adsorbed surface compared to TCPS controls.

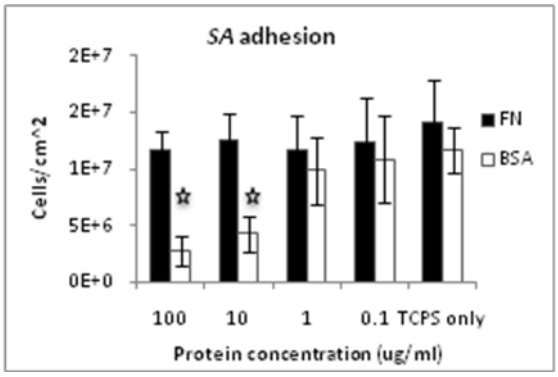

Figure 2. *SA* binding to FN and BSA via specific and non-specific adhesion. * indicates significant ($p<0.05$) difference between BSA adsorbed surface compared to TCPS controls.

Conclusions: *SE* and *SA* bind to FN by very different mechanisms. Whereas *SA* has FN specific receptors, *SE* binds via non-specific adhesion and is unable to differentiate between FN and BSA coated surfaces. A single method to deter bacterial adhesion to protein coated surfaces may appear efficient when testing against one species of bacteria. However, even closely related bacteria have differing mechanisms surface attachment and careful examination of the anti-adhesive nature of such surfaces is necessary.

References: (National Center for Health Statistics. Center for Disease Control. 1999:119: 99.)